UNIVERSITY OF PENNSYLVANIA

THE INFLUENCE OF SEVERAL BENZENE DERIVATIVES ON THE ROOTS OF LUPINUS ALBUS

MOTHER MARY CHRYSOSTOM

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

PHILADELPHIA
1936

THE INFLUENCE OF SEVERAL BENZENE DERIVATIVES ON THE ROOTS OF LUPINUS ALBUS

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF PENNSYLVANIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

MOTHER MARY CHRYSOSTOM

Reprint from
The American Journal of Botany, Vol. 23, No. 7

PHILADELPHIA
1936

R. UNIV.
BIBLIOTHEEK
LEIDEN

Reprinted from the American Journal of Botany, Vol. 23, No. 7, 461–471, July, 1936
Printed in U. S. A.

THE INFLUENCE OF SEVERAL BENZENE DERIVATIVES ON THE ROOTS OF LUPINUS ALBUS [1]

Mother Mary Chrysostom

Benzene substitution products have long afforded a very attractive field of research to the chemist and bacteriologist and, more recently, to the plant pathologist. To the chemist, the ease of preparation of these substances and the evident correlation between their chemical properties and their structure present almost unlimited scope for investigation. The bacteriologist early discovered their antiseptic value and is now following the chemist in testing the newer derivatives for possible use as antiseptics and germicides. Literature in this field is voluminous and constantly being augmented. Owing to the fact that various plant products are benzene derivatives oftentimes formed in the course of destructive metabolism, the plant pathologist is beginning to consider their presence in the tissue as the active principle of immunity to certain diseases. Such investigations have stimulated research on the use of many of these substances as fungicides and germicides.

There is a decided paucity of literature, however, on the benzene compounds in relation to the higher plants. True and Hunkel (1898) investigated the influence of several phenols, aromatic acids, and their salts on *Spirogyra* and the roots of *Lupinus albus*. They concluded that the toxic effect of the benzene nucleus was modified to a greater or less degree by the introduction of various radicals into it. Brown and Tinker (1916a, 1916b), in their studies on the permeability of the seed coats of *Hordeum vulgare*, found that various phenols were rapidly absorbed. Brenchley (1918), in a study of the effects of phenols on barley and peas with the possible use of these substances for soil sterilization, found that high concentrations were very toxic to the roots, while weak concentrations did not seem to retard growth. Recently, Eisenmenger (1931) studied the effect of silica gel in modifying the toxicity of phenol to soy bean

[1] Received for publication April 3, 1936.

The writer wishes to express her deep gratitude to Professor Rodney H. True for suggesting the problem and for his unfailing interest during the course of the work, and to Doctor John R. Skeen for his kind assistance in reading the manuscript.

seedlings. It would seem then that a quantitative study of their influence might be of interest.

In their studies on the penetration of various inorganic substances into plant tissue, True and Bartlett (1915), Osterhout (1922), and Stiles and Jørgensen (1914) have measured the electrical conductance of the tissue itself or of the solutions bathing the tissues as an indication of changes in permeability of the membranes. The method gives a fair quantitative measure of increase of electrolytes in the tissue or in the liquid surrounding it. Thus, to consider only the solutions bathing the roots, if conductance increases there has been exosmosis of electrolytes from the tissue; if it decreases there has been absorption from the solution with or without exosmosis of electrolytes. As a result of his study on exosmosis of electrolytes from potato tissue, Stiles (1927) concluded that increase in electrolytes accompanied by evident turbidity might be considered the result of injury to the membrane and therefore a rough measure of toxicity. With this in mind electrical conductance of the solutions at constant time intervals will be compared as a measure of exosmosis from the roots; that is, of the toxic effect of the solution.

The solutions studied are of three classes: (a) the acidic—the phenols; (b) the more strongly acidic—the acids; (c) the weak bases—the amines. Consequently a comparative study of changes in hydrogen-ion concentration of the solutions has been made. To correlate the effects noted, the physiological condition of the roots was observed.

Experimental material.—*Seeds.*—Seeds of *Lupinus albus* were selected and grown by the usual method in chopped sphagnum until the primary roots were between 60 and 70 mm. in length. This stage of growth was reached in about three days. Seedlings were washed free of sphagnum in distilled water and dried with absorbent paper. The seed coats were then carefully removed, and the seedlings were placed in culture jars. These consisted of 500-cc. beakers covered by the lid of a Petri dish which was pierced in four places to allow the roots to go through. Each seedling was fixed in place with absorbent cotton.

Solutions.—All the solutions used in this study were made volumetrically from stock solutions of C.P. chemicals. The cresol purchased was a U.S.P. mixture of ortho-, para-, and meta-cresol. The water used was redistilled from Pyrex glass and its conductance varied between 1 and 2×10^{-6} mhos. As it was not desired to kill the roots immediately, a series of preliminary experiments was performed using at least ten concentrations of the solutions. As a result, the following concentrations were chosen for phenol, cresol, resorcinol, pyrogallol, aniline, methyl aniline: 20×10^{-4} M, 8×10^{-4} M, and 2×10^{-4} M. For benzoic, salicylic, and gallic acids, 16×10^{-5} M, 8×10^{-5} M, and 2×10^{-5} M were used.

Seedlings were also grown in a standard nutrient solution and in distilled water for purposes of comparison.

Methods and procedure. — Resistances were read on a Leeds and Northrup conductivity apparatus and recorded as mhos.

Hydrogen-ion readings were made by the quinhydrone titration method using McIlvaine's standard buffers—citric acid and disodium hydrogen phosphate.

All cultures were kept in the dark in a constant temperature room. During one series of experiments the temperature of the solutions remained at $23 \pm 1°$C., and during the series performed at what is designated "high" temperature at $28 \pm 1°$C.

The conductance and hydrogen-ion concentrations of the solutions were taken, and the roots were fixed in position immediately. At the end of one hour conductance was taken by removing the roots to an empty beaker and inserting a dip electrode in the solution. The roots were replaced immediately. In all solutions except that of the acids there was a slight increase in conductance, but this was not significant enough to be incorporated in the curves. The acids revealed a slight decrease. Then conductances were taken at regular time intervals of 24 hours for eight days. At the same periods hydrogen-ion concentrations were read on a 10 cc. sample taken from the culture jars. This sample was replaced by ten cc. of the original solution plus a quantity of water necessary to bring the level back to 500 cc.

A second similar series was studied, but the samples taken from the culture jars for hydrogen-ion readings were boiled for one minute, cooled to original temperature, and then titrated. It was hoped to drive off CO_2 by this procedure.

RESULTS

For this presentation it is desirable to treat the effect of the solutions in three specific groups: (1) the phenols; (2) the aromatic acids; (3) the amines. Under each separate heading will be considered conductance, hydrogen-ion concentration, and physiological effects. As rate is more interesting in this study than gross change, graphs are plotted as semilog—i.e., log conductance against constant time intervals of twenty-four hours. All values have been corrected to 20°C.

Phenols. — *Conductance.* — Figures 1, 2, 3, and 4 show the effects of the four phenols used on the conductance of the solutions. It will be noted that the general shape of the curves is the same: A very great increase in conductance during the first twenty-four hours is followed by a gradual increase during the rest of the experiment. Pyrogallol differs from the other solutions in that its initial increase is extended over a period of two days and that the increase for the remainder of the experiment is much greater per day than that of the other solutions. Except in the case of cresol and pyrogallol, concentration makes very little difference. In the two solutions mentioned the strongest concentration produced the greatest change, and this was decidedly more marked than in the other two.

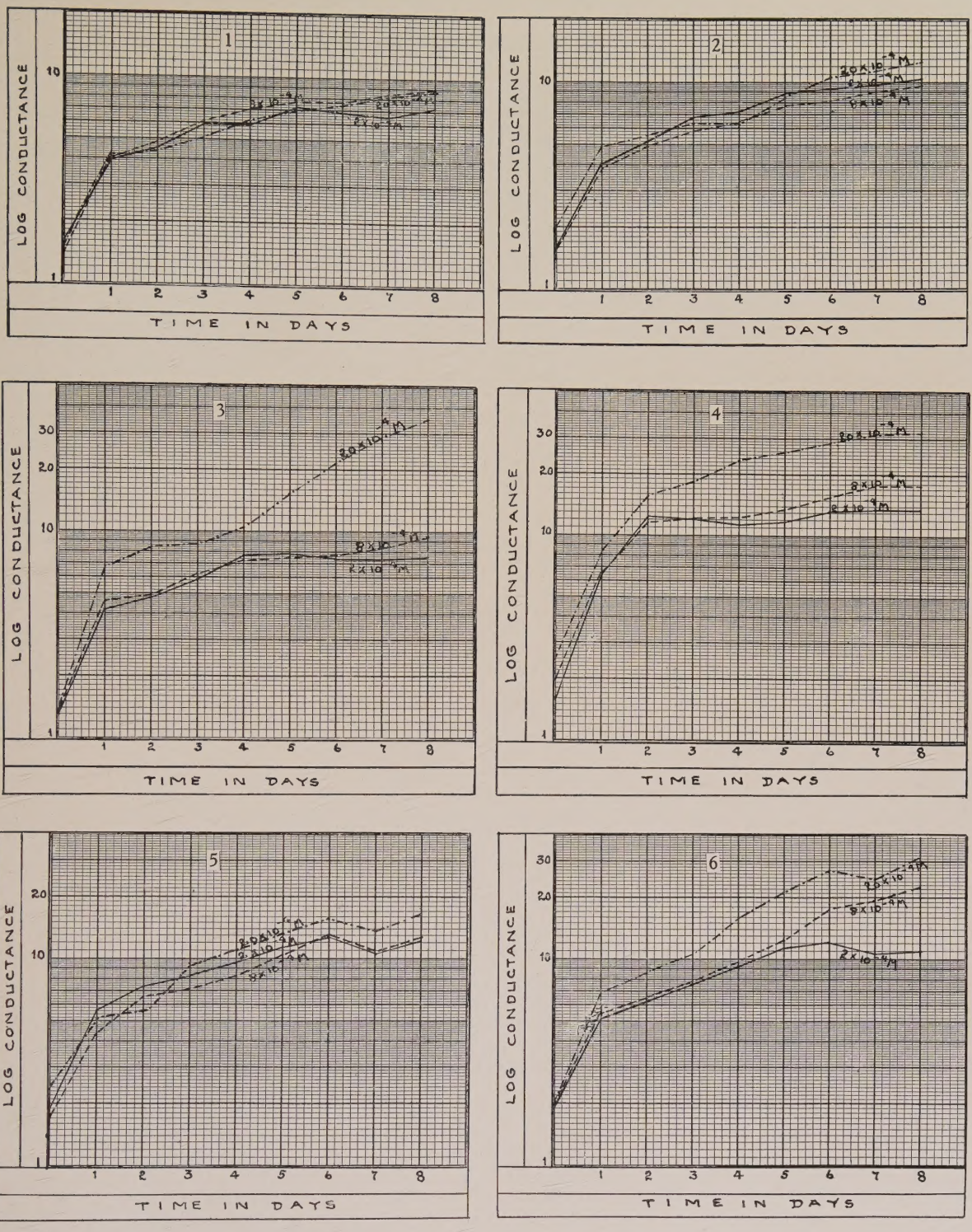


Fig. 1–6.—Changes in conductance of solutions.—Fig. 1. Phenol (23°C.).—Fig. 2. Resorcinol (23°C.).—Fig. 3. Cresol (23°C.).—Fig. 4. Pyrogallol (23°C.).—Fig. 5. Phenol (28°C.).—Fig. 6. Cresol (28°C.).

TABLE 1. *Hydrogen-ion concentration.*[a]

	Phenol						Cresol						Resorcinol					
	$2x10^{-4}M$		$8x10^{-4}M$		$20x10^{-4}M$		$2x10^{-4}M$		$8x10^{-4}M$		$20x10^{-4}M$		$2x10^{-4}M$		$8x10^{-4}M$		$20x10^{-4}M$	
	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.
Original	5.6	5.6	5.4	5.4	5.5	5.5	5.5	5.5	5.4	5.4	5.5	5.2	5.3	5.5	5.4	5.4		5.5
1st Day	5.4	4.9	5.4	4.8	5.2	4.8	5.4	5.1	5.5	5.2	5.7	5.4	5.8	5.7	6.3	5.6		5.6
2nd Day	5.4	6.1	5.2	6.1	5.2	6.3	5.6	5.9	5.4	6.1	5.7	6.2	5.5	5.8	5.6	6.0		5.6
3rd Day	5.2	5.9	5.5	5.8	5.5	6.0	5.1	6.1	5.2	5.9	5.4	5.9	5.7	6.5	5.6	6.5		6.6
8th Day	5.5	6.1	5.4	6.4	5.2	6.1	5.1	6.0	5.4	6.6	6.4	6.6	5.5	6.4	5.6	6.3		6.4

	Pyrogallol						Benzoic						Salicylic					
	$2x10^{-4}M$		$8x10^{-4}M$		$20x10^{-4}M$		$2x10^{-5}M$		$8x10^{-5}M$		$16x10^{-5}M$		$2x10^{-5}M$		$8x10^{-5}M$		$16x10^{-5}M$	
	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.
Original	4.8	4.8	4.6	4.6	4.4	4.4	5.0	5.0	4.3	4.3	3.8	3.8	5.7	5.7	4.9	4.9	4.2	4.2
1st Day	5.2	4.5	5.1	4.8	5.2	5.4	5.8	5.7	5.6	4.8	5.8	4.6	5.8	5.9	4.9	4.9	4.2	4.2
2nd Day	5.6	5.0	5.6	5.5	4.7	4.3	5.7	6.2	5.6	6.0	5.7	5.7	5.6	6.2	5.5	6.3	5.7	6.2
3rd Day	5.6	5.4	5.2	5.6	4.6	4.6	5.8	6.0	5.6	6.0	5.7	6.3	6.0	6.2	6.4	6.1	6.4	6.1
8th Day	6.3	6.3	5.5	5.7	5.7	4.8	5.8	6.4	5.8	6.5	5.8	6.4	6.2	6.6	6.1	6.4	6.1	6.6

	Gallic						Aniline						Methyl aniline					
	$2x10^{-5}M$		$8x10^{-5}M$		$16x10^{-5}M$		$2x10^{-4}M$		$8x10^{-4}M$		$20x10^{-4}M$		$2x10^{-4}M$		$8x10^{-4}M$		$20x10^{-4}M$	
	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.	pH U.	pH B.
Original	4.8	4.8	4.3	4.3	4.0	4.0	5.1	5.1	5.4	5.4	5.8	5.8	5.6	5.6	5.7	5.7	6.0	6.0
1st Day	5.2	5.6	4.5	4.7	4.3	4.7	5.5	5.9	5.6	6.0	5.8	6.2	5.6	6.3	5.3	6.2	5.6	6.3
2nd Day	5.5	5.5	5.4	5.4	5.6	5.2	5.5	6.1	5.5	6.3	5.8	6.2	5.6	6.3	5.3	6.2	5.6	6.3
3rd Day	5.3	6.5	5.2	6.2	5.4	6.1	5.3	6.3	5.3	6.3	5.5	6.4	5.5	6.3	5.4	6.3	5.8	6.5
8th Day	5.7	6.2	5.8	6.2	5.7	6.1	5.7	7.1	6.2	7.3	5.1	7.2	6.0	7.2	6.3	7.5	6.8	7.3

[a] U means unboiled; B means boiled.

A closer study reveals the further fact that phenol and resorcinol are quite similar in initial effect, but considerably different in end results; cresol causes a greater increase during the first twenty-four hours than phenol or resorcinol.

Temperature effects.— Figures 5 and 6 show the results of the series of experiments conducted with phenol and cresol at a temperature of 28°. In all cases except the most concentrated cresol solution, there is a greater exosmosis from the roots than at the lower temperature. This may account for the increase noted in the graphs.

In general, then, a study of the changes in conductance of these solutions reveals that: (1) There was an increase in conductance in all concentrations; (2) this increase in conductance was greatest for all solutions during the first twenty-four hours; (3) there was no marked difference as a result of concentration except in the strongest cresol and pyrogallol solutions; (4) exosmosis from the roots increased in the following order: phenol, resorcinol, cresol, pyrogallol; (5) exosmosis was greater from the roots at 28° into phenol and cresol solutions than at the lower temperature.

Hydrogen-ion concentrations.—Two sets of readings are given for each solution. The first, designated as unboiled, is the reading as it was taken on the solutions immediately after they were removed from the culture jars. The second, designated as boiled, is the reading taken on a sample which had been boiled for one minute. In presenting the first set of readings, it is recognized that the roots are constantly throwing off CO_2 into the solutions and that the quantity has been shown to be greatest during the first twenty-four hours and to gradually fall to a minimum (Åslander, 1933). Thus the amount present must tend to equilibrium. They are expected therefore to be more acid. It was hoped that boiling would drive off the CO_2 formed. It would seem, however, that during boiling other changes may have taken place at least in some of the solutions — e.g., those which became distinctly more acid during the first twenty-four hours and remained acid even after boiling. Readings are presented for the first three days and for the end of the experiment.

PHENOL.—Considering the unboiled solutions first, it will be noticed that there is a tendency to greater acidity, a fact which would be expected. But there is no neutralizing action in these solutions and all end more acid or as acid as the original. This may be due to increased respiration with consequent evolution of more carbon dioxide than normal.

In the boiled solutions there is a definite drop to much greater acidity at the end of twenty-four hours.

The contrast here with the unboiled solutions is so great that there must have been more changes in the solutions than driving off CO_2. Perhaps the roots have excreted some other volatile compound which remains undisturbed in the unboiled, but is driven off in boiling. Prianischnikow (1928) attributed the neutralizing action of roots in culture solutions to NH_3 production. Subsequently there is a sharp increase to 6.1 or 6.3, and all solutions end considerably more basic than they began.

Cresol.—There is considerable variation in the unboiled solutions. With some fluctuations, the solu-

Table 2. *Physiological effects of the phenols.*

	Roots					Cotyledons	Epicotyl
	Primary root		Laterals				
Phenol	Tip	Mature region	Time of appearance	Character	Length		
$2x10^{-4}M$	Flaccid 5th day	Thick and knotted	1st day	Scattered	3–4 cm.	Normal	Growth
$8x10^{-4}M$	Flaccid 4th day	Thick and knotted	1st day	Scattered bunched at top	3–4 cm.	Normal	Growth
$20x10^{-4}M$	Flaccid 4th day	Thick and knotted	1st day	Bunched at top	2 cm.	Normal	Growth
Cresol							
$2x10^{-4}M$	Flaccid 3rd day	Normal	3rd day	Scattered	3–4 cm.	Slight separation	No growth
$8x10^{-4}M$	Flaccid attenuated 2nd day	Normal	3rd day	Bunched at top	2 cm.	Slight separation	No growth
$20x10^{-4}M$	Flaccid attenuated 1st day	Flaccid 4th day	3rd day	Bunched few along roots	1 cm.	Slight separation	No growth
Resorcinol							
$2x10^{-4}M$	Flaccid 3rd day	Normal	1st day	Normal	4–5 cm.	Normal	Slight growth
$8x10^{-4}M$	Flaccid 2nd day	Normal	1st day	Normal	4–5 cm.	Normal	Growth
$20x10^{-4}M$	Flaccid 2nd day	Normal	1st day	Bunched at top	4–5 cm.	Normal	Growth
Pyrogallol							
$2x10^{-4}M$	Flaccid 1st day	Flaccid	1st day	Few	1–2 cm.	Normal	Growth
$8x10^{-4}M$	Flaccid 1st day	Flaccid	1st day	Few at top	1 cm.	Slight separation	No growth
$20x10^{-4}M$	Flaccid 1st day	Flaccid	3rd day	Bunched at top	No growth	No separation	No growth
Benzoic Acid							
$2x10^{-5}M$	Flaccid 3rd day	Normal	3rd day	Scattered	3–4 cm.	Normal	Growth
$8x10^{-5}M$	Flaccid 2nd day	Normal	3rd day	Scattered	3–4 cm.	Normal	Growth
$16x10^{-5}M$	Flaccid 1st day	Normal	3rd day	Aggregated at top	3–4 cm.	Normal	Very little growth
Salicylic Acid							
$2x10^{-5}M$	Flaccid 3rd day	Flaccid	1st day	Scattered	3 cm.	Slight separation	Slight growth
$8x10^{-5}M$	Flaccid 2nd day	Flaccid	1st day	Aggregated at top	3 cm.	Slight separation	Slight growth
$16x10^{-5}M$	Flaccid 1st day	Flaccid	3rd day	Whorled	4 cm.	No separation	No growth

TABLE 2. *Continued.*

	Roots					Cotyledons	Epicotyl
	Primary root		Laterals				
GALLIC ACID	Tip	Mature region	Time of appearance	Character	Length		
$2x10^{-5}M$	Flaccid 4th day	Normal	1st day	Normal along whole length	3–4 cm.	Normal	Growth
$8x10^{-5}M$	Flaccid 2nd day	Normal	1st day	Normal whole length	3–4 cm.	Normal	Growth
$16x10^{-5}M$	Flaccid 2nd day	Normal	1st day	Normal half of region	3–4 cm.	Normal	Growth
ANILINE							
$2x10^{-4}M$	Flaccid 5th day	Normal	2nd day	Scattered bunched at top	1–2 cm.	Slight separation	Slight growth
$8x10^{-4}M$	Flaccid 4th day	Flaccid ½ length	2nd day	Bunched at top	1 cm.	Slight separation	No growth
$20x10^{-4}M$	Flaccid 3rd day	Flaccid whole length	2nd day	Very small bunches	1 cm.	Slight separation	No growth
METHYL ANILINE							
$2x10^{-4}M$	Flaccid 2nd day	Flaccid whole length	2nd day	———	———	No separation	———
$8x10^{-4}M$	Flaccid 2nd day	Flaccid whole length	2nd day	———	———	No separation	———
$20x10^{-4}M$	Flaccid 1st day	Flaccid whole length	2nd day	———	———	No separation	———

tions reach their most acid condition on the third day. The two lower concentrations end more acid than the original or just the same, while the highest concentration becomes as basic as the boiled solutions.

In the boiled solutions except for the first drop in acidity there is an almost unbroken progression to a more basic condition. Thus phenol and cresol may be looked upon as having practically the same effect in stimulating changes of hydrogen-ion concentration.

RESORCINOL. — Results in the strongest unboiled solution are not available, owing to unavoidable mishaps. In the two weakest concentrations there is a progression with much fluctuation to more basic solutions. After boiling there is an almost unbroken sequence to pH of 6.3 and 6.4.

PYROGALLOL. — Original solutions were much more acid than any of the phenols. There is a more gradual change to a more basic solution in all cases. The strongest boiled solution ends decidedly acid. Since pyrogallol changes chemically in air and on boiling, other factors are affecting hydrogen-ion readings, and neutralization is probably the resultant of many factors.

Physiological effects. — The physiological effects of the phenols are summarized in table 2. It will be noted that the strongest cresol solution and all pyrogallol solutions show marked toxic effects. In these solutions the root tips became flaccid almost immediately and this condition extended to the mature region of the root. Laterals were tardy in appearing in the cresol solutions and in the most concentrated pyrogallol solution. Subsequent development consisted in an aggregation of very stunted laterals at the top of the roots. Seedlings in these solutions showed very little top development.

In the phenol and resorcinol solutions, development was more or less normal.

Turbidity appeared at about the same time in all solutions.

AROMATIC ACIDS.—*Conductance.*—Curves for these results are presented in figures 7, 8, and 9. The striking contrast between these graphs and those of the phenols is very evident. Considering the three acids together, there is, in general, a decrease in conductance followed by an increase and a final levelling, except in the highest concentration of salicylic acid.

Benzoic acid. — In the lowest concentration, the initial decrease in conductance extends over a period of twenty-four hours, in the next concentration over a period of forty-eight hours, and in the highest concentration, ninety-six hours. After this period, conductance increases until the sixth day, when all curves show a constant level until the end of the experiment. It will be noted that there is very little increase in the strongest solution, more in the intermediate and most in the weakest. Except in the

latter solution, leach does not compensate for initial absorption.

Salicylic acid.—Decrease in conductance in salicylic acid solutions is similar to that in benzoic acid in point of time, but much greater in amount. In the weakest concentration there is a decrease for twenty-four hours, in the intermediate for forty-eight hours, and in the strongest for one hundred twenty hours. The two lower concentrations then increase in conductance until the sixth and seventh day, when they remain at the same level. The highest concentration gradually rises in conductance until the last day, when it shows a very sharp increase. Only the weakest solution reaches a conductance greater than the original value.

Gallic acid.—This acid produces much less effect than the other two. There is no decrease in conductance in the weakest concentration, a slight decrease for forty-eight hours in the intermediate, and for seventy-two hours in the highest. The lowest concentration then gives the most increase, while the two higher increase slightly for two and three days, respectively, and then remain at comparatively the same level.

In all of the acid solutions only the weakest concentrations reach a final conductance which is greater than that of the original solutions. It would seem, then, that whatever ions were removed from the solution by the plants were not compensated for completely by the end of the experiment. In order of increasing effect these acids would be arranged as follows: gallic, benzoic, salicylic.

In general, graphs for the acids show: (1) A decrease in conductance at first for varying periods, seemingly dependent on concentration; (2) a very decided concentration effect; (3) a final increase in conductance above its initial point.

Hydrogen-ion concentration.—Changes in hydrogen-ion concentration for the three acids may be presented in a concisely comparative study. In all solutions there is a distinct neutralization reaction on the part of the roots under the influence of which the solutions end much more basic than they were originally.

Unboiled solutions.—It will be observed from table 1 that the greatest change in the hydrogen-ion concentration of all the acids takes place during the first forty-eight hours. From then to the end of the experiment there is a progressive tendency to a more neutral end point with only slight variations. This point is the same for all concentrations of each acid—e.g., 5.8 for benzoic, 6.1 for salicylic, and 5.7 for gallic. The initial change in acidity can be correlated with decrease in conductance during the first two days.

Boiled solutions.—Except for the fact that all readings are more basic than those of the unboiled, the results seem to be the same as in the latter solutions. The striking change in acidity during the first twenty-four hours in the unboiled solutions seems to require at least forty-eight hours in the case of benzoic and salicylic acids and seventy-two hours in the case of gallic. End points for the solutions of each acid are the same—e.g., 6.4 or 6.5 for benzoic, 6.4 or 6.6 for salicylic, and 6.2 or 6.1 for gallic.

Physiological effects.—If the effects listed in table 2 are studied, it will be noted that salicylic acid is more toxic than either benzoic or gallic. There is a softening of the entire root in all the concentrations with no apparent elongation. In the most concentrated solution laterals have developed, but are whorled in arrangement since there was no accompanying elongation of the root. In the same solution cotyledons did not separate and there was no growth of the epicotyl.

Benzoic acid is next greatest in toxic effect on the roots. Tips are flaccid the first day and laterals are late in appearing and then scattered or aggregated at the top of the region of growth. Tops show development in all the solutions.

Gallic acid seems to have less effect than the other two. Only the root tips seem affected and the region of side root growth is shorter in the strongest concentration.

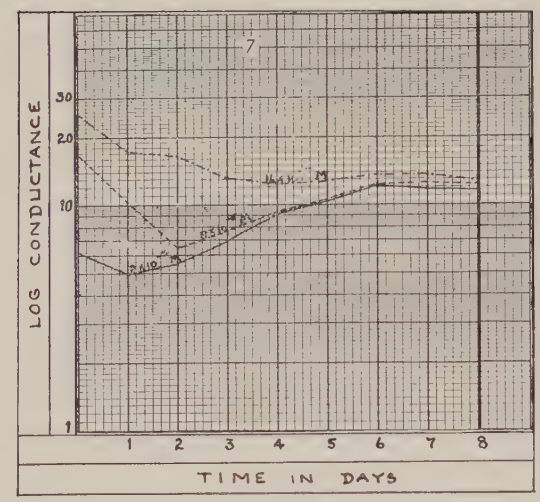
All solutions were turbid from about the third day.

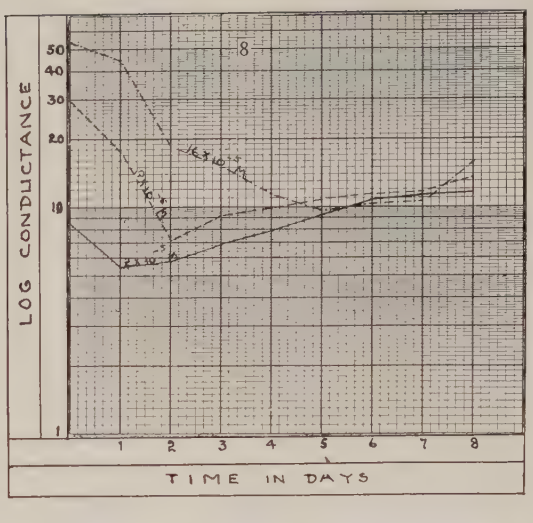
In order of increasing toxicity the solutions are: gallic acid, benzoic acid, salicylic acid.

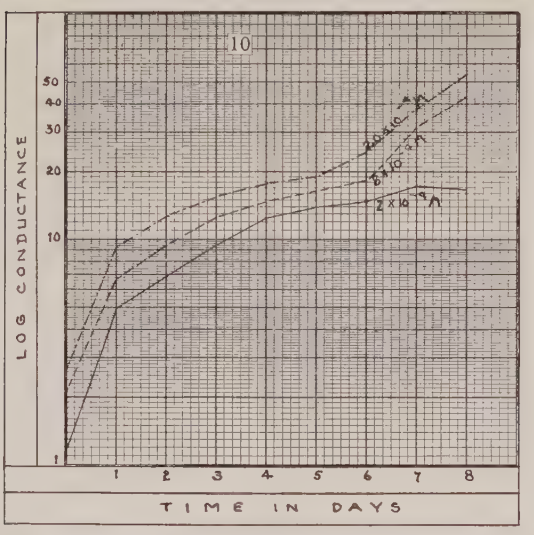
THE AMINES.—*Conductance.*—Figures 10 and 11 show changes in conductance of the solutions of the amines. In general, there is a very great increase in conductance; much greater than in the phenols. The rate of change in both solutions is greatest during the first twenty-four hours. Concentration of these solutions seems to have a much greater effect on the roots than that of any other substance studied. Using the amount of exosmosis of electrolytes as a criterion, methyl aniline is much more toxic than aniline.

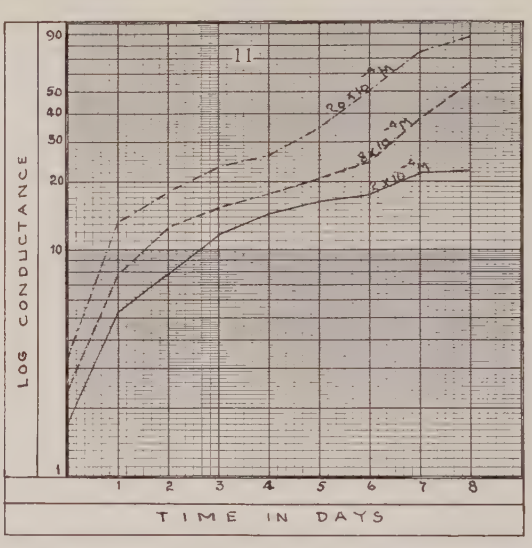
Temperature 28°.—Just as with phenol and cresol, higher temperature causes a greater increase in conductance in the solutions. With both substances the curves are very much steeper than those at the lower temperature. It will be noted that the curve for the strongest methyl aniline solution leveled at the end of the fourth day. This was probably due to the fact that roots in solutions of that strength very quickly reached a state of disintegration. Perhaps the equilibrium established at that point is an indication of death. No other solution reaches equilibrium so early in the experiment.

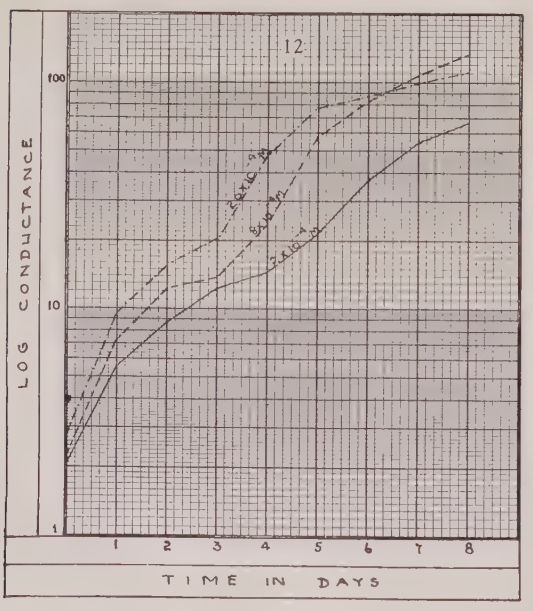
HYDROGEN-ION CONCENTRATION.—With the generalization that the boiled solutions are very much more basic than the unboiled, the two sets of solutions may be considered together. The two unboiled solutions of methyl aniline became slightly more acid the first day. This was probably due to the carbon dioxide factor. The other solutions changed steadily in the direction of neutrality. End points for all these solutions are more basic than any yet considered. The readings for the first three days and for the last day are included in table 1.

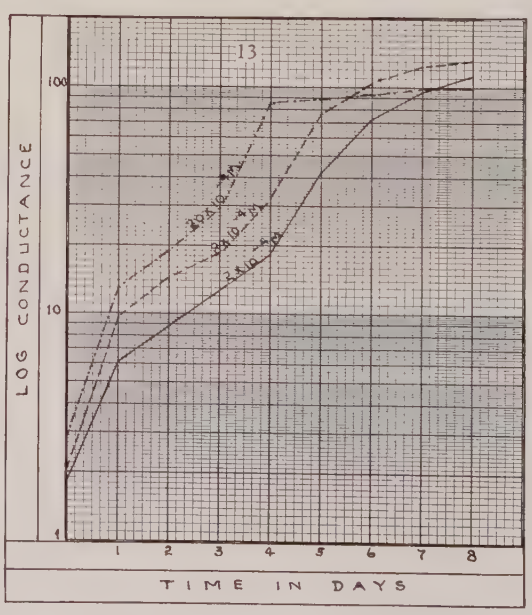

7
LOG CONDUCTANCE
30
20
10
1
1 2 3 4 5 6 7 8
TIME IN DAYS


8
LOG CONDUCTANCE
50
40
30
20
10
1
1 2 3 4 5 6 7 8
TIME IN DAYS


10
LOG CONDUCTANCE
50
40
30
20
10
1
1 2 3 4 5 6 7 8
TIME IN DAYS


11
LOG CONDUCTANCE
90
50
40
30
20
10
1
1 2 3 4 5 6 7 8
TIME IN DAYS


12
LOG CONDUCTANCE
100
10
1
1 2 3 4 5 6 7 8
TIME IN DAYS


13
LOG CONDUCTANCE
100
10
1
1 2 3 4 5 6 7 8
TIME IN DAYS

Physiological effects.—The results listed in table 2 point to the fact that these solutions are much more toxic than any of the others studied with the possible exception of pyrogallol. Root tips became flaccid slightly later than in the aromatic acids, but this softening was progressive, except in the weakest aniline solution. Finally the whole root was affected. Laterals grew to some extent in the two weaker aniline solutions but not at all in the strongest except in small aggregations at the top of the root. In methyl aniline solutions, laterals were inhibited immediately on formation. Tops showed a slight growth in aniline, but none in methyl aniline. The loss of turgidity in the latter solutions extended throughout the seedlings.

NUTRIENT.—*Conductance.*—The nutrient solution showed a decrease in conductance for the first five days, then an increase. This is in accordance with what might be expected, since the plants are constantly taking ions from the solutions. The increase at the end of the experiment was probably caused by unequal exchange of ions.

Hydrogen-ion concentration.—These solutions became progressively more acid, even those which were boiled to remove the carbon dioxide. Evidently the cause of acidity in the unboiled as well as the boiled solutions was not due entirely to carbon dioxide.

Physiological effects.—In the lupines grown in the nutrient solution there was a plentiful growth of lateral roots and a decided elongation of the primary root. Hypocotyls elongated, the cotyledons opened, and there was considerable growth of the epicotyl. Except for the yellow color of the leaves, the plants could be considered in good condition. The roots were turgid and the solutions clear at the end of the experiment.

DISTILLED WATER.—*Conductance.*—The conductance of distilled water in which plants have grown increases in an almost constant ratio from day to day. This effect is in accordance with the work of various investigators who attribute the rise to electrolytes diffusing from the roots.

Hydrogen-ion concentration.—These readings show very little difference from those already discussed. There is a neutralizing tendency much more marked in the boiled than in the unboiled solutions. Few fluctuations are noted in the readings from day to day.

Physiological effects.—Plants grown in distilled water, although not normal in development, were in very good condition in comparison with those grown in toxic solutions. There was a decided elongation of the primary root with a scattered growth of laterals. These, however, attained considerable length. At the close of the experiment the tips had become soft. Some of the hypocotyls showed clearing and had collapsed at the end of the time, the primary bud opened and the stem elongated slightly. Solutions did not become turbid until the fifth day.

DISCUSSION

From the results presented, it is very evident that exosmosis from the roots of the seedlings studied is a result of cell injury which ultimately results in death. The materials diffusing from the roots were electrolytes, since they caused an increase in the conductance of the solution, and colloidal substances, since all solutions showed a persistent turbidity. The toxic substances may have first attacked the surface layer of the cells, making it permeable to electrolytes and other substances to which it would normally be impermeable. This explanation has been advanced by Stiles and Jørgensen (1917) as a result of their study of exosmosis from roots into organic solutions. On the other hand, the toxic substance may have been absorbed by the roots, and attacking the cytoplasm, may have caused the formation of easily diffusible substances. This explanation has been given by Brooks (1916) to his results from studies on exos-

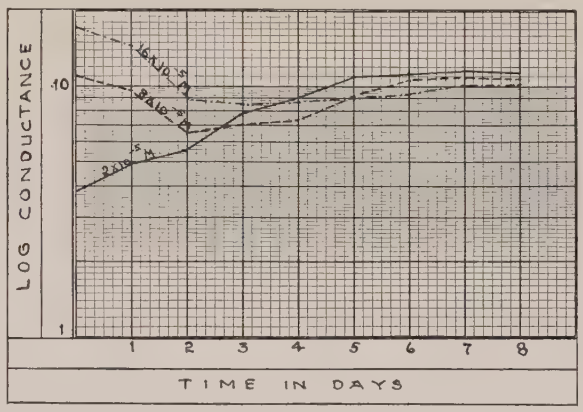


Fig. 9. Changes in conductance of gallic acid solutions (23°C.).

mosis from roots. The present study seems to present evidence for both explanations. In the concentration of the phenols studied, the effects on the roots seem to be purely local, permitting good top development, but killing the root tips almost immediately. Phenols are known to precipitate and coagulate proteins as well as other labile colloidal systems. If the external surface of the cytoplasm is colloidal, as many investigators believe, the first effect of the solutions would be external, but there would then be a gradual attack on the cytoplasm which would finally kill. Pyrogallol killed much more rapidly than the other solutions. It is more soluble and more sensitive to oxidation than the other phenols used. Its penetration was more rapid and its effect more evident.

The question arises as to what factor caused toxicity. The acids used in this study are only slightly dissociated, so that there are in the solution hydrogen-ions and a great number of undissociated

Fig. 7, 8, 10, 11, 12, 13.—Changes in conductance of solutions.—Fig. 7. Benzoic acid (23°C.).—Fig. 8. Salicylic acid (23°C.).—Fig. 10. Aniline (23°C.).—Fig. 11. Methyl aniline (23°C.).—Fig. 12. Aniline (28°C.).—Fig. 13. Methyl aniline (28°C.).

molecules. The hydrogen-ions cannot be the only toxic agent. If they were, benzoic and gallic acids would be more toxic than salicylic, but this contradicts the results. It seems that the evidence here is in favor of undissociated molecules as the principal toxic factor. This opinion has been advanced by True (1900) for *Lupinus albus*, by Clark (1899) for filamentous fungi, by Collett (1919) for *Paramoecium* and *Euplotes*, and has been held by many other investigators.

The amines were most devastating in their action. The toxic effect was general. They are rapidly absorbed and rapidly transported to all parts of the seedlings. They seem to produce a general disintegration of the protoplasm with very great leach, greater than in any of the solutions in spite of the much smaller absorbing surface exposed. They are both water-soluble and fat-soluble, a fact which may account for their rapid penetration. Rapidity of penetration of weak bases and aliphatic amines into cells has been reported by Harvey (1911) for animals and plants and by Brooks (1923) for plants. This investigation points to a similar characteristic of the aromatic amines studied.

Hydrogen-ion concentrations recorded for this study cannot be considered significant except in the acid solutions. In these the evident agreement between fall in pH and decrease in conductance points to the absorption of hydrogen-ions as the mechanism of neutralization. In the other solutions, with the exception of pyrogallol, the decrease in pH cannot be attributed to the absorption of hydrogen-ions as there is no evidence to support such a hypothesis. Rather, the fact of exosmosis of colloidal substances from the roots points to a combination of these substances with the hydrogen-ions of the solution to form more slightly dissociated compounds. Hydrogen-ions in the solution would therefore become fewer. This explanation has been advanced by Mevius (1927) in his studies of root growth in various solutions.

This investigation shows a correlation between the physiological action and the structure of the molecule in both the phenol series and the amine series. Cresol, which is formed by the substitution of a methyl group for one of the hydrogen atoms of the nucleus, is more toxic than phenol. True and Hunkel (1898) noted this fact as a result of their studies on *Lupinus albus*, and Woodward, Kingery, and Williams (1934) as a result of their studies on fungi. With the phenols having increasing number of hydroxyl groups—namely, phenol, resorcinol, and pyrogallol—there is a correlation similar to that found by Walker and Link (1935) in their studies with onion bulb parasites. When the hydroxyl groups are in the ortho position, there is an increase in toxicity — namely, pyrogallol is more toxic than phenol; when the hydroxyl groups are in the meta position, there is little or no increase in toxicity—namely, resorcinol is less toxic than phenol or as toxic. It is interesting to add that salicylic acid, the most toxic of the acids studied, has the hydroxyl group in the ortho position. Among the amines a methyl group in the aniline series increased toxicity. This has been observed to be true for bacteria also by Bachman (1928).

SUMMARY

A study has been made of the effects of several benzene derivatives on the roots of *Lupinus albus*—namely, four phenols (phenol, cresol, resorcinol, and pyrogallol); three acids (benzoic, salicylic, and gallic); two amines (aniline and methyl aniline).

The effects have been expressed in changes of conductance and hydrogen-ion concentrations of the solutions as well as in the physiological influence on the plants.

In general, all solutions increased in conductance except the acids, which decreased for varying periods of time and then increased. Except in the lowest concentrations of the acids, the ions lost to the solutions were not compensated for by the end of the experiment.

Hydrogen-ion concentration became considerably less in all solutions except the most concentrated of the pyrogallol solutions.

There seems to be an agreement between toxicity and complexity of structure in all of these compounds. Among the phenols studied, the order of increasing toxicity is phenol, resorcinol, cresol, pyrogallol. Among the acids studied, the order is gallic, benzoic, and salicylic, and among the amines, aniline and methyl aniline.

There is some indication that rise in temperature increases the toxic effect in the case of phenol, cresol, aniline, methyl aniline.

University of Pennsylvania,
Philadelphia, Pennsylvania

LITERATURE CITED

Åslander, A. 1933. Neutralizing action of plants on acid nutrient solutions. Svensk. Bot. Tidskr. 27: 257–272.

Bachman, G. W. 1928. Chemical constitution and germicidal action. Proc. Soc. Exp. Biol. and Med. 25: 249–250.

Brenchley, W. E. 1918. Organic plant poisons. II. Phenol. Annals Bot. 32: 259–278.

Brooks, M. M. 1923. Studies on the permeability of living and dead cells. III. The penetration of certain alkalies and ammonium salts into living and dead cells. Pub. Health Rep. 866, p. 2074–2086.

Brooks, S. C. 1916. Studies on exosmosis. Amer. Jour. Bot. 3: 483–492.

Brown, A. J., and F. Tinker. 1916a. Rate of absorption of various phenolic solutions by the seeds of *Hordeum vulgare* and the factors governing the rate of diffusion of aqueous solutions across semi-permeable membranes. Proc. Roy. Soc. London B, 89: 119–135.

———, and F. Tinker. 1916b. Selective permeability. The absorption of phenol and other solutions by the seeds of *Hordeum vulgare*. Proc. Roy. Soc. London B, 89: 373–379.

Clark, J. F. 1899. On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. 28: 289–327, 378–404.

Collett, M. E. 1919. Toxicity of acids to ciliate infusoria. I, II. Jour. Exp. Zool. 29: 443–472.

Eisenmenger, W. S. 1931. Factors modifying the toxicity of phenol. Plant Physiol. 6: 325–332.

Harvey, E. N. 1911. Studies in the permeability of cells. Jour. Exp. Zool. 10: 507–556.

Mevius, W. 1927. Kalzium Ion und Wurzelwachstum. Jahrb. Wiss. Bot. 66: 183–253.

Osterhout, W. J. V. 1922. Injury, recovery and death in relation to conductivity and permeability. Monographs on Experimental Biology 8: 1–259. Philadelphia and London.

Prianischnikow, D. 1928. Über die Ausscheidung von Ammonia durch Pflanzenwurzeln der Saurevergiftung. Biochem. Zeitschr. 193: 211–215.

Stiles, W. 1927. Exosmosis of dissolved substances from storage tissue into water. Protoplasma 2: 577–601.

———, and I. Jørgensen. 1914. The measurement of electrical conductivity as a method of investigation in plant physiology. New Phytol. 13: 226–242.

———, and I. Jørgensen. 1917. Studies in permeability. IV. The action of various organic substances on the permeability of the plant cell and its bearing on Czapek's theory of the plasma membrane. Annals Bot. 31: 47–76.

True, R. H. 1900. The toxic action of a series of acids and of their sodium salts on *Lupinus albus*. Amer. Jour. Sci. 9: 183–192.

———, and H. H. Bartlett. 1915. Exchange of ions between the roots of *Lupinus albus* and culture solutions containing two nutrient salts. Amer. Jour. Bot. 2: 311–323.

———, and C. G. Hunkel. 1898. The poisonous effects exerted on living plants by phenols. Bot. Centralbl. 75, 76: 289–295, 321–327, 391–398.

Walker, J. C., and K. Link. 1935. Toxicity of phenolic compounds to certain onion bulb parasites. Bot. Gaz. 96: 468–484.

Woodward, G. I., L. B. Kingery, and R. J. Williams. 1934. The fungicidal power of phenol derivatives. I. Effect of introducing alkyl groups and halogens. Jour. Lab. and Clin. Med. 19: 1216–1223.